Biomaterials Developed with an Oxyresveratrol-Rich Artocarpus lakoocha Extract Exhibiting High Phenolic and Flavonoid Contents, Strong Antioxidant Activity, and In Vitro Fibroblast Compatibility

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Abstract

Aim: Wounds pose significant clinical challenges due to delayed healing and a high risk of infection. Advanced biomaterials that provide structural support and are enriched with bioactive compounds offer promising solutions. The aim of this study was to determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH) of biomaterials enriched with extracts derived from Artocarpus lakoocha (A. lakoocha), which were identified as Oxyresveratrol-rich based on LC–MS Q-TOF phenolic profiling; and to evaluate the effects of these biomaterials on cytotoxicity, cell adhesion, and cytoskeletal organization using NIH-3T3 fibroblast cells in vitro.

Methods: Extracts of A. lakoocha were prepared and analyzed for TPC, TFC, antioxidant activity using the DPPH assay, and phenolic profiling by LC–MS Q-TOF. Biomaterials enriched with the extract were fabricated using an appropriate synthesis approach. Cytocompatibility was evaluated in NIH-3T3 cells by MTT assay at 24, 48, and 72 hours, and immunofluorescence imaging was performed to examine cell adhesion and cytoskeletal organization.

Results: The A. lakoocha extract exhibited high phenolic (541.3 ± 14.1 mg GAE/g) and flavonoid (96.3 ± 5.1 mg QE/g) contents, along with strong antioxidant activity (IC_{50} = 98.03 ± 0.57 µg/mL). LC–MS Q-TOF analysis revealed Oxyresveratrol as the predominant phenolic compound in the extract. Extract-enriched biomaterials exhibited porous structures with high surface area and maintained excellent swelling capacity (>415%). The MTT assay demonstrated a significant increase in cell viability, particularly at 48 hours (approximately 12.9% increase). Immunofluorescence imaging revealed enhanced F-actin organization and improved intercellular interactions.

Conclusions: Biomaterials enriched with A. lakoocha extract and rich in Oxyresveratrol demonstrated favorable physicochemical properties, strong antioxidant activity, and superior cytocompatibility, indicating strong potential as advanced wound dressings. Further in vivo studies are recommended to confirm therapeutic efficacy.

Keywords: *Wound dressing; Oxyresveratrol; Artocarpus lakoocha; antioxidant activity; biomaterial; antioxidant activity; fibroblast compatibility*

1. Introduction

The skin, as the body's primary protective shield, functions as a physiological barrier that safeguards the organism against environmental threats[1]. Skin barrier impairment can result from both intrinsic factors such as genetic mutations in filaggrin and extrinsic influences including excessive hygiene practices, low humidity, and exposure to surfactants. Repeated mechanical or chemical irritation can disrupt the lipid matrix and corneocyte cohesion, thereby weakening the barrier function and increasing susceptibility to dermatological disorders[2]. Maintaining the integrity of the skin barrier is essential, as it provides effective defense against microbial invasion, allergens, and chemical irritants[3]. Moreover, impaired barrier function has been associated with the pathogenesis of various dermatological conditions[4]. Globally, approximately 2% of hospitalized patients suffer from chronic skin wounds, including lacerations, burns, and pressure injuries underscoring the substantial need for effective wound management strategies[5]. Appropriate wound care accelerates healing and reduces recurrence and complications. Despite advances, conventional wound dressings often fail to adequately address complex healing scenarios due to limited adaptability, poor moisture regulation, and insufficient support for regenerative cell activity.

Due to their unique characteristics, including high flexibility, pronounced hydrophilicity, superior absorbency, and soft consistency, hydrogels have emerged as promising materials for advanced wound dressing applications[6]. These three-dimensional polymeric networks can be formulated from a wide range of natural and synthetic polymers, either individually or in combination. Synthetic polymers commonly used for hydrogel wound dressings in-

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clude poly(ethylene glycol) (PEG), polyacrylamide (PAM), and polyvinyl alcohol (PVA), which provide mechanical stability and tunable swelling properties[7]. Natural polymers, such as chitosan, collagen, alginate, gelatin, hyaluronic acid, and cellulose derivatives, offer inherent biocompatibility and biodegradability, making them suitable for biomedical applications[8,9]. These hydrogels can be further functionalized with therapeutic agents, nanoparticles, or bioactive plant extracts to enhance their antimicrobial, anti-inflammatory, or pro-regenerative capabilities[10,11]. Despite these advantages, conventional hydrogels often exhibit limitations, such as relatively low mechanical strength, restricted pore size, and limited oxygen and nutrient diffusion, which can compromise their performance in deep or chronic wound environments[12,13]. To overcome these drawbacks, cryogels have emerged as promising alternatives. During cryogelation, polymerization occurs at subzero temperatures, resulting in the formation of interconnected macroporous networks characterized by rapid swelling kinetics, high permeability, and enhanced mechanical resilience. These characteristics make cryogels highly suitable for applications in wound healing, tissue engineering, and drug delivery[14,15].

Artocarpus lakoocha Roxb. (A. lakoocha), a medicinal plant belonging to the Moraceae family, is predominantly distributed in tropical regions of Southeast Asia, India, and Thailand[16,17]. Commonly known as "monkey jack," this species has been widely used in traditional medicine for the treatment of various ailments including inflammation, diarrhea, abscesses, and parasitic infections. Numerous studies have reported the pharmacological activities of extracts obtained from different parts of *A. lakoocha*, such as the leaves, bark, and heartwood, revealing antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and anticancer properties[18,19,20]. The major active compound of *A. lakoocha* is Oxyresveratrol, a polyphenolic stilbene with high antioxidant capacity, which has been shown to support wound healing by promoting fibroblast proliferation, collagen synthesis, and the upregulation of wound-related gene expressions such as VEGFR-2 and TGF-β1[21,22,23,24]. Additionally, in vitro studies have demonstrated that ethanolic extracts of *A. lakoocha* significantly suppress LPS-induced inflammatory cytokines including IL-6, TNF-α, and MCP-1 in macrophage cell lines, suggesting its strong anti-inflammatory effects via modulation of PI3K/Akt and NF-κB pathways[25,26]. The antibacterial efficacy of *A. lakoocha* has been validated against several wound-associated pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, exhibiting average inhibition zones ranging from 10–20 mm[27]. In the present study, *A. lakoocha* extract was incorporated into PVA/collagen-based cryogels, and comprehensive physicochemical characterization and in vitro biological evaluations were conducted to assess their potential suitability as wound dressing materials. Specifically, this study aimed to determine the total phenolic content (TPC), total flavonoid content (TFC), phenolic profile (LC–MS Q-TOF), and antioxidant activity (DPPH) of the extract-enriched biomaterials, as well as to evaluate their effects on cytotoxicity, cell adhesion, and cytoskeletal organization using NIH-3T3 fibroblast cells in vitro.

2. Materials and Methods

2.1. Total Phenolic Content (TPC)

The total phenolic content of *A. lakoocha* extracts was determined using the Folin–Ciocalteu colorimetric method described by Singleton and Rossi (1965), with some modifications[28]. The *A. lakoocha* sample (100 µL) was mixed with 1 mL of deionized water and 250 µL of Folin-Ciocalteu's reagent solution and 150 µL 7% (v/v) sodium carbonate (Na_2CO_3) solution. The mixture was incubated at room temperature for 45 min. The absorbance was measured at 750 nm using spectrophotometer (Shimadzu, Kyoto, Japan). The amount of TPC gallic acid equivalent (GAE) in the extract samples was calculated as "mg GAE/g dry extract". The experiments were performed in triplicate.

2.2. Total Flavonoid Content (TFC)

The total flavonoid content of *A. lakoocha* extracts was determined using the Aluminum Chloride Colorimetric Method reported by Zhishen[29]. The *A. lakoocha* sample (100 µL) was mixed with 300 µL of 30% ethanol and 200 µL of 10% aluminium chloride ($AlCl_3$) solution. The mixture was incubated for 10 minutes. After 10 min of incubation, 200 µL of 1 M sodium acetate (NaOAc) and 1 ml of H2O were added and incubated for 60 min. After that, the absorbance values of the samples were measured using a spectrophotometer at a wavelength of 415 nm. A calibration curve was created using a quercetin standard to calculate the total flavonoid content. A calibration curve was prepared with quercetin standard. The results were expressed as mg QE/g extract. The experiments were performed in triplicate.

2.3. DPPH Radical Scavenging Activity

The antioxidant capacity of the extracts was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method described by Blois, with some modifications[30]. For analysis, volumes of extract solutions prepared at different concentrations were taken and mixed with a 0.1 mM DPPH solution prepared in methanol. After the mixtures were homogenized, reactions were carried out in the dark due to the light sensitivity of DPPH, and the samples were incubated at room temperature for 30 minutes. The absorbance was measured at 517 nm by a spectrophotometer. IC_{50} values were determined by nonlinear regression using GraphPad Prism 9.1.0 software. Butylated hydroxytoluene (BHT) served as a positive control. All experiments were performed in triplicate, and data were presented as mean ± standard deviation (SD). The radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.4. LC–MS Q-TOF Analysis for Chemical Profiling

The metabolite profile of *A. lakoocha* extracts was analyzed using LC–MS Q-TOF system (Agilent Technologies, USA). Chromatographic separation was performed on an Agilent Poroshell C18 column (3.0 × 100 mm, 2.7 µm). Two solvents, one containing 0.1% formic acid, and the other acetonitrile, were used as mobile phases during the analysis. The flow rate was set at 0.6 mL/min. Mass spectrometric data were acquired in both positive and negative electrospray ionization modes with these parameters: capillary voltage, 2000 V; drying gas (N_2), 11 L/min at 325 °C; nebulizer pressure, 35 psi; and collision energies of 10, 20, and 30 eV. Metabolite identification was performed using Agilent MassHunter software, supported by METLIN, PubChem, FooDB databases, and MS/MS fragmentation pattern analysis.

2.5. Synthesis of Artocarpus lakoocha Extract–Enriched Polymeric Cryogels

A 10% (w/v) poly(vinyl alcohol) (PVA) solution (Sigma-Aldrich, USA) was prepared by dissolving PVA in ultrapure water at 90 °C under constant stirring. Separately, a 1% (w/v) type I collagen solution (fish-derived, Sigma-Aldrich, USA) was prepared at 40 °C. The two solutions were then mixed at a 1:2 (v/v) ratio. Subsequently, *A. lakoocha* extract, characterized by an Oxyresveratrol-rich phenolic profile, was incorporated into the polymer mixture to obtain a final extract concentration of 1% (w/v). Glutaraldehyde (Merck, Germany) was added as a cross-linking agent, and the mixture was gently homogenized and immediately dispensed into 24-well plates. For comparison, control cryogels without *A. lakoocha* extract were prepared following the same protocol. The samples were frozen at −16 °C for 12 h to induce cryogelation.

2.6. Swelling Tests

Cryogels were dried with a lyophilizer (BK-FD10, Biobase) at −55 °C for 3 h. Dried cryogels were weighed (m_d) and immersed in phosphate-buffered saline (PBS) at 37 °C. At predetermined intervals, the swollen cryogels were weighed (m_{sw}). The swelling ratio (SR) was calculated using the following equation:

$$SR\ (\%) = (m_{sw} - m_d) / m_d \times 100 \quad \text{(Equation 1)}$$

2.7. BET analysis

Specific surface area and porosity characteristics of the cryogels were evaluated using the Brunauer–Emmett–Teller (BET) method with nitrogen adsorption–desorption isotherms at 77 K[31]. Prior to analysis, samples were dried under vacuum at 40 °C for 24 h to remove residual moisture. The specific surface area of the samples was determined based on the volume of nitrogen adsorbed and expressed in units of m^2/g.

2.8. Cell Culture Studies

NIH-3T3 human fibroblast cells were cultured in RPMI-1640 medium (Sigma-Aldrich, Germany) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 100 U/mL penicillin–streptomycin under standard conditions (37 °C, 5% CO_2). Prior to cell culture, *A. lakoocha* extract–enriched cryogels were sterilized by immersion in a graded ethanol series prepared at 60%, 70%, 80%, 90%, and 100% (v/v) for a total of 5 minutes at each stage[32]. For cell seeding, NIH-3T3 cells were placed on the biomaterials (13 mm diameter, 0.5 mm thickness) at a density of 1×10^6 cells for each sample.

2.9. MTT Assay

The MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a colorimetric technique that determines cell viability based on the reduction of the MTT reagent by metabolically active mitochondria in viable cells[33]. MTT assay was performed at 24, 48 and 72 hours. The experimental setup was a positive control group (cells cultured without cryogel) and a negative control group (cryogel without cells). MTT solution (0.5 g/mL) was added to each well and the cells were incubated at 37 °C and 5% CO_2 for 3 h. After incubation, the medium was removed and dimethyl sulfoxide (DMSO) was added and incubated for 30 min. The absorbance values were measured with a spectrophotometer (Shimadzu, Uvmini-1240) at a wavelength of 570 nm. Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (\text{Mean Abs570 of test group} / \text{Mean Abs570 of control group}) \times 100$$

In this formula, the "test group" refers to cells cultured on *A. lakoocha* extract–enriched cryogels, while the "control group" refers to cells cultured directly on the plate without any biomaterials[32,34,35].

2.10. Immunofluorescence analyses

Immunofluorescence analysis was performed to evaluate the effects of *A. lakoocha* extract–enriched cryogels on the morphology of NIH-3T3 and on cell-cell and cell-matrix interactions. For this purpose, biomaterials samples were placed in 24-well culture plates and seeded with NIH-3T3 cells. The cells were cultured under incubation conditions for 48 hours. Following incubation, biomaterials were fixed in PBS containing 4% (v/v) paraformaldehyde for 30 minutes at room temperature. After fixation, samples were washed with PBS to remove residual fixatives. Cell were permeabilized using 0.1% (v/v) Triton X-100 in PBS for at 10 minutes. F-actin filaments were stained using Alexa Fluor 594-conjugated phalloidin, prepared in PBS containing 1% (w/v) bovine serum albumin (BSA) and Tween-20 (PBS-T), at a dilution of 1:1000. For nuclear staining, 4′,6-diamidino-2-phenylindole (DAPI) was diluted 1:1000 in PBS and applied simultaneously. The staining solutions were incubated with the samples for 90 minutes in the dark at room temperature. After incubation, samples were washed with PBS and imaged using a fluorescence microscope (Dmil LED Fluo, Leica, Germany).

2.11. Phase Contrast Microscopy

To examine cell morphology on biomaterials, 13 mm diameter Thermanox™ coverslips (Thermo Fisher Scientific, USA) were placed on 24-well culture plates, and then cryogels samples were fixed on these coverslips. After 48 h of incubation, the samples were examined using a phase-contrast microscope (Leica DMiL LED Fluo, Germany).

2.12. Statistical Analysis

All statistical analyses were performed using SPSS software (version 20.0; IBM, Chicago, IL, USA). All findings are expressed as the mean values and standard deviation (SD) of three independent replicates. GraphPad Prism software (v8.4.3, GraphPad Software Inc., USA) was used for advanced comparisons and graphical presentations. Fluorescence intensity measurements were quantified using ImageJ software. For comparisons involving three or more groups, two-way ANOVA followed by Tukey's post-hoc test was applied. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Determination of Total Phenolic and Flavonoid Contents and Assessment of Antioxidant Potential in Artocarpus lakoocha

The evaluation of *A. lakoocha* revealed a high content of phenolic and flavonoid compounds, along with substantial antioxidant capacity (Table 1). The total phenolic content (TPC), determined using the Folin–Ciocalteu method, was 541.3 ± 14.1 mg GAE/g extract, indicating a strong presence of phenolic constituents. The total flavonoid content (TFC), assessed by the aluminum chloride colorimetric assay, was 96.3 ± 5.1 mg QE/g extract, further supporting the phytochemical richness of the extract. Antioxidant activity was measured by DPPH radical scavenging assay and demonstrated an IC_{50} value of 98.03 ± 0.57 µg/mL, reflecting significant free radical neutralizing ability. Compared to previous studies reporting TPC values ranging from 127.99 to 398.44 mg GAE/g and TFC values between 2.05 and 8.79 mg QE/g in ethanol-based extracts, the significantly higher levels observed in this study may be attributed to differences in extraction methodology, solvent polarity, and plant material[19,36]. The IC_{50} value aligns with literature data reporting antioxidant activities within the range of ≤50–100 µg/mL for *A. lakoocha* extract assessed through DPPH and other radical scavenging assays[37,38,39,40]. These findings confirm the phytochemical richness and potent antioxidant potential of *A. lakoocha* extract, highlighting its promise as a functional ingredient in biomaterials aimed at enhancing wound healing.

Table 1

Phytochemical Composition and Antioxidant Activity of Artocarpus lakoocha Extract

Parameter	Measurement (n=3)	Unit
TPC	541.3±14.1	mg GAE/g extract
TFC	96.3 ± 5.1	mg QE/g extract
DPPH IC_{50}	IC50: 98.03 ± 0.57	µg/mL

3.2. Main Phenolic Compounds Identified in Artocarpus lakoocha by LC–MS Q-TOF

Analysis revealed that Oxyresveratrol, Resveratrol, and Gallic acid were the primary bioactive phenolic compounds in the extract. Quantitative assessment revealed that Oxyresveratrol was present at the highest concentration, at 78.8 ± 9.1 µg/mg of extract. This was followed by gallic acid (9.2 ± 0.9 µg/mg) and resveratrol (4.5 ± 0.6 µg/mg) (Table 2). These values were calculated as approximately 78.8, 9.2, and 4.5 mg/g, respectively, on a dry weight basis, demonstrating that the extract is quite rich in phytochemicals.

Table 2

Quantitative Analysis of Major Phenolic Compounds in Artocarpus lakoocha Extract

Compound Name	Retention Time (RT, min)	$[M-H]^-$ Ion (m/z)	Concentration (µg/mg extract)
Oxyresveratrol	5.62	243.1	78.8 ± 9.1
Resveratrol	6.04	227.1	4.5 ± 0.6
Gallic Acid	2.45	169.0	9.2 ± 0.9

3.3. Morphological and Structural Characteristics of Artocarpus lakoocha Extract–Enriched Cryogels

Cryogels incorporating *A. lakoocha* extract were successfully synthesized using a PVA/collagen blend through the cryogelation process. The resulting biomaterials exhibited a macroporous structure with well-interconnected pores, as confirmed by visual inspection (Figure 1). Cryogels enriched with *A. lakoocha* extract, characterized by an Oxyresveratrol-rich phenolic profile, displayed a light yellow coloration. Importantly, the incorporation of the extract did not adversely affect the cryogel formation process. Both control and extract-enriched biomaterials maintained their three-dimensional structural integrity after repeated freeze–thaw cycles, indicating good structural stability.

Figure 1

Macroscopic view of cryogels prepared with A. lakoocha extract

3.4. Characterization Studies

Swelling ratio analysis demonstrated that cryogels incorporating *A. lakoocha* extract, characterized by an Oxyresveratrol-rich phenolic profile, exhibited a slightly reduced water uptake capacity compared to control cryogels. This minor reduction can be attributed to additional hydrogen-bonding interactions between phenolic functional groups present in the extract and the polymer matrix. Despite this decrease, the extract-enriched cryogels retained excellent hydration capacity. The swelling ratio of the control cryogel was 420 ± 3.2%, whereas cryogels enriched with *A. lakoocha* extract exhibited a swelling ratio of 415 ± 4.5%. Brunauer–Emmett–Teller (BET) analysis revealed distinct surface and porosity characteristics for the extract-enriched cryogels, indicating the formation of a highly porous network suitable for biomedical applications. The specific surface area of the control cryogel was determined to be 12.8 ± 0.9 m^2/g, while cryogels incorporating *A. lakoocha* extract exhibited a higher specific surface area of 15.2 ± 2.1 m^2/g. This increase suggests an expanded surface area that may facilitate improved cell adhesion. All BET measurements were performed in triplicate, and the results are presented as mean ± standard deviation. Overall, the incorporation of *A. lakoocha* extract with an Oxyresveratrol-rich phenolic profile led to a modest increase in specific surface area without compromising the hydration properties of the cryogels.

3.5. MTT Assay

The effects of control cryogels and cryogels incorporating *A. lakoocha* extract, characterized by an Oxyresveratrol-rich phenolic profile, on cell proliferation were evaluated using the MTT assay at 24, 48, and 72 hours. As shown in Table 3, compared with the control group (100%), cell proliferation in the control cryogel group was reduced by 3.8% ± 2.1%, 10.4% ± 0.8%, and 12.5% ± 1.4% at 24, 48, and 72 hours, respectively. These reductions were statistically significant at 48 and 72 hours, whereas no significant difference was observed at 24 hours. In contrast, cryogels incorporating *A. lakoocha* extract exhibited a significant proliferative effect. Cell proliferation increased by 8.7% ± 2.2%, 12.9% ± 2.2%, and 10.3% ± 2.4% at 24, 48, and 72 hours, respectively, compared to the control group ($p < 0.0001$). Overall, these findings indicate that while the control cryogel slightly suppressed cell proliferation over time, the incorporation of *A. lakoocha* extract with an Oxyresveratrol-rich phenolic profile significantly enhanced cell viability in a time-dependent manner, with the most pronounced effect observed at 48 hours (Figure 2, Table 3).

Figure 2

Cell viability of NIH-3T3 fibroblast cells cultured on control cryogels and A. lakoocha extract–enriched cryogels at 24, 48, and 72 hours. Cell proliferation was assessed using the MTT assay, and data are presented as mean ± standard deviation (n = 3).

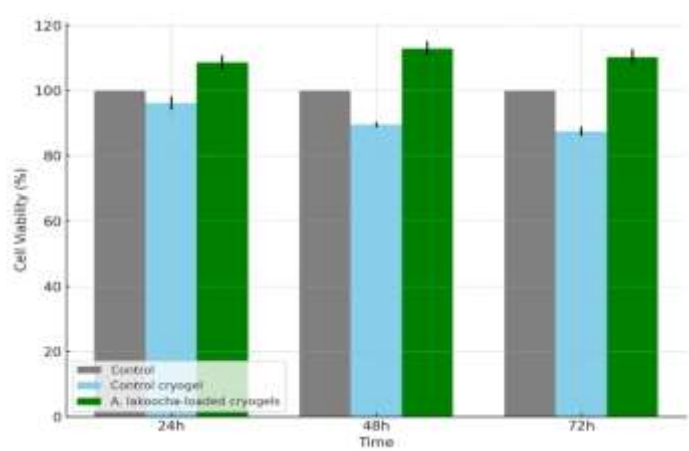


Table 3

Cell Viability of NIH-3T3 Cells on Cryogels at 24, 48, and 72 Hours

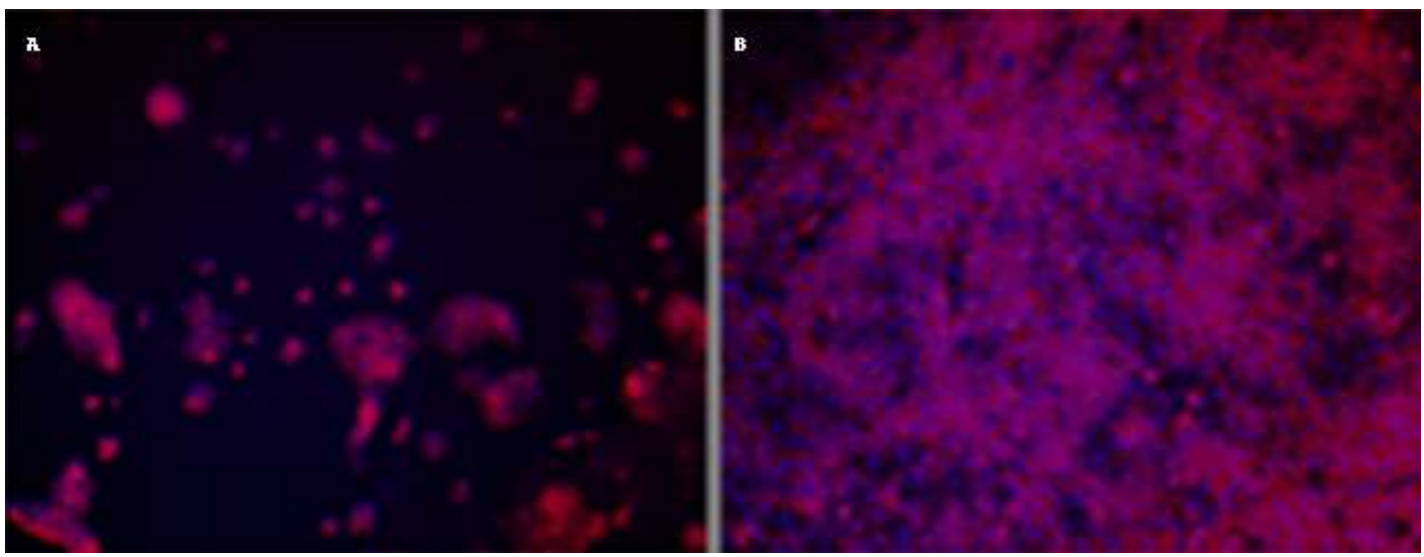
Group	24 h Viability (%)	48 h Viability (%)	72 h Viability (%)
Control	100	100	100
Control cryogel	96.2 ± 2.1	89.6 ± 0.8	87.5 ± 1.4
A. lakoocha extract–enriched cryogels	108.7 ± 2.2	112.9 ± 2.2	110.3 ± 2.4

3.6. Immunofluorescence Analysis of NIH-3T3 Cells on Artocarpus lakoocha Extract–Enriched Cryogels

Immunofluorescence imaging demonstrated that NIH-3T3 fibroblast cells adhered to and spread effectively on both control cryogels and cryogels incorporating *Artocarpus lakoocha* extract after 48 hours of incubation (Figure 3). F-actin staining (red) revealed well-organized cytoskeletal structures, with cells exhibiting typical fibroblast morphology. Notably, cells cultured on extract-enriched cryogels, characterized by an Oxyresveratrol-rich phenolic profile, displayed more pronounced actin filament organization and extensive lamellipodia formation, indicating enhanced cell–substrate interactions and increased migratory potential compared to control cryogels. DAPI staining (blue) showed a uniform distribution of nuclei throughout the cryogel matrix, suggesting a high density of viable cells. Furthermore, cells cultured on *A. lakoocha* extract–enriched cryogels formed more interconnected cellular networks, reflecting enhanced cell–cell communication and a favorable microenvironment for tissue integration. Overall, these findings indicate that *A. lakoocha* extract–enriched cryogels effectively support fibroblast attachment, proliferation, and cytoskeletal organization, which are critical parameters for wound healing applications.

Figure 3

Immunofluorescence images of NIH-3T3 fibroblast cells cultured on cryogel matrices after 48 hours. (A) Control cryogel and (B) *Artocarpus lakoocha* extract–enriched cryogel. F-actin filaments were stained with phalloidin (red), and cell nuclei were counterstained with DAPI (blue), demonstrating cell adhesion, spreading, and cytoskeletal organization within the cryogel microarchitecture.



4. Discussion

The findings obtained in the study indicate that the extract of *A. lakoocha* plant has high phytochemical content and strong antioxidant potential. The total phenolic content was determined as 541.3 ± 14.1 mg GAE/g using the TPC assay, while the total flavonoid content was measured as 96.3 ± 5.1 mg QE/g extract using the TFC assay. These values determined by the used experimental methods were significantly higher than the literature studies, where values in ethanol-based extracts of *A. lakoocha* plant were found to be 127.99 - 398.44 mg GAE/g by the TPC method and 2.05 - 8.79 mg QE/g by the TFC method[19,36]. Such discrepancies between the results obtained from the current study and those from literature studies may be attributed to differences in experimental methods, such as differences in extraction methods and solvent polarity, as well as differences in the geographical origin of the plants used and harvesting conditions[10,41]. The findings of this study suggest that the use of more polar solvents and optimized extraction protocols, including longer extraction times than those used in previous studies, may have increased the yield of the bioactive compounds present[42,43].

Consistent with its elevated phenolic and flavonoid content, the extract exhibited substantial antioxidant activity, as demonstrated by the DPPH radical scavenging assay with an IC_{50} value of 98.03 ± 0.57 µg/mL. This result indicates a strong free radical neutralizing capacity and is consistent with previously reported IC_{50} values for *A. lakoocha* extracts, which typically range from ≤50 to 100 µg/mL[37,38,39,40]. Antioxidant activity is generally correlated with phenolic content due to the electron-donating properties of phenolic hydroxyl groups, which can stabilize free radicals[44,45]. There-

fore, the high antioxidant efficacy observed in this study is likely associated with the extract's elevated phenolic and flavonoid composition[46].

Furthermore, LC–MS Q-TOF analysis revealed that the most abundant phenolic compound in the extract was Oxyresveratrol (78.8 ± 9.1 µg/mg), followed by gallic acid (9.2 ± 0.9 µg/mg) and resveratrol (4.5 ± 0.6 µg/mg). These compounds are well known for their antioxidant and anti-inflammatory activities, which may collectively contribute to the overall bioactivity profile of the extract[47,48]. Notably, Oxyresveratrol, a Resveratrol analogue with additional hydroxyl groups, has been reported to exhibit strong antioxidant activity and potential relevance for skin-related application in various studies, making its high presence in *A. lakoocha* particularly relevant for biomedical applications, including wound healing[49,50].

In summary, the high levels of phenolic and flavonoid compounds, coupled with potent antioxidant activity and the presence of bioactive metabolites such as Oxyresveratrol and Gallic acid, underscore the potential of *A. lakoocha* extract as a valuable natural source for therapeutic applications. These results support its further development as a functional ingredient in antioxidant-rich formulations, particularly for use in biomaterials aimed at promoting tissue repair and enhancing wound healing outcomes[51,52,53].

A. lakoocha extract extract-enriched cryogels were successfully synthesized using a PVA/Collagen blend via a freeze–thaw cryogelation technique. The fabrication process resulted in macroporous cryogels with interconnected structures, visually confirming the integrity and uniformity of the formed network. Control and extract-enriched cryogels maintained their three-dimensional structure after repeated freeze–thaw cycles[54,55].

Swelling analysis revealed that incorporation of *A. lakoocha* extract led to a slight reduction in water absorption capacity compared to control cryogels (415 ± 4.5% vs. 420 ± 3.2%). This decrease, although minimal, may be attributed to additional hydrogen-bonding interactions between phenolic hydroxyl groups present in the extract and functional groups within the polymer matrix such as –OH groups in PVA and amide (–NH–CO–) and carboxyl (–COOH) groups in type I collagen[54,56,57]. Such interactions likely reduced the number of available hydrophilic sites capable of binding water molecules, leading to a modest decrease in swelling behavior. Nevertheless, the extract–enriched polymeric cryogels preserved excellent hydration capacity, a desirable feature for wound dressing applications where moisture retention is essential for maintaining a moist healing environment and promoting tissue regeneration[52,55].

BET analysis provided further insights into the structural properties of the cryogels. Interestingly, the specific surface area of cryogels increased upon *A. lakoocha* incorporation (from 12.8 ± 0.9 m^2/g in the control to 15.2 ± 2.1 m^2/g in the extract–enriched cryogels), suggesting that the addition of the extract may contribute to changes in porosity and surface roughness[56,58]. This increase in surface area is of particular relevance for biomedical applications, as it may facilitate improved cell adhesion, nutrient exchange, and bioactive compound diffusion across the cryogel network[59,60]. The presence of phenolic compounds could influence pore formation during cryogelation by altering ice crystal nucleation and growth dynamics, thus subtly modulating the internal microarchitecture of the resulting biomaterial[31,58]. Overall, the physicochemical characterization results support the suitability of *A. lakoocha*-enriched cryogels as promising candidates for biomedical applications, particularly in wound healing. Their favorable swelling behavior, enhanced surface area, and stable macroporous structure suggest that these cryogels can effectively support cell–material interactions while simultaneously delivering antioxidant and bioactive molecules derived from the extract.

The MTT assay results revealed a distinct difference in cellular responses between control cryogels and *A. lakoocha*-enriched cryogels over the 72-hour evaluation period. Control cryogels exhibited a slight reduction in cell proliferation compared to the baseline, which may be attributed to limited nutrient diffusion and initial cell adaptation to the three-dimensional macroporous structure[13,15]. This decrease is consistent with previous reports indicating that synthetic or semi-synthetic cryogels, despite their interconnected porosity, can transiently restrict metabolic activity during early incubation phases due to surface chemistry and hydrophilicity variations[14]. Conversely, the incorporation of *A. lakoocha* extract markedly enhanced cell viability at all time points, with the most pronounced effect observed at 48 hours. This finding is consistent with previous studies reporting the bioactive potential of phenolic and flavonoid compounds present in *A. lakoocha*, which exhibit antioxidant and anti-inflammatory activities conducive to cellular proliferation and wound healing[31,38]. Polyphenolic constituents such as Oxyresveratrol and stilbene derivatives, previously identified in *A. lakoocha*, are known to modulate oxidative stress pathways and promote cell survival through upregulation of pro-regenerative signaling cascades. These mechanisms may contribute to the observed enhancement in cell proliferation.

Immunofluorescence analysis provided valuable insights into the cytocompatibility and morphological behavior of NIH-3T3 cultured on cryogel matrices. Immunofluorescence imaging further corroborated the MTT results by demonstrating enhanced cell adhesion and spreading on *A. lakoocha*-enriched cryogels. Both control and extract–enriched cryogels supported fibroblasts attachment; however, cells on extract–enriched cryogels exhibited more pronounced F-actin organization and extensive lamellipodia formation, indicative of stronger substrate interactions and enhanced migratory potential[14]. Cytoskeletal integrity and lamellipodia extension are critical determinants of fibroblasts motility, a process essential for re-epithelialization during wound healing[61]. The improved cytoskeletal integrity on *A. lakoocha*-integrated cryogels may be attributed to the bioactive phytochemicals, such as Oxyresveratrol and flavonoids, known for their antioxidant and anti-inflammatory properties[38]. These compounds can modulate oxidative stress and inflammatory signaling, thereby promoting cell survival and enhancing actin dynamics[31]. Additionally, the formation of interconnected cellular networks observed in the extract-loaded group suggests improved cell–cell communication and tissue-like organization within the macroporous cryogel matrix[11]. These findings reflect the synergistic influence of cryogel microarchitecture, which supports oxygen and nutrient diffusion, and the bioactivity of incorporated phytochemicals that modulate cellular signaling[12,14]. Future studies should further investigate the molecular pathways regulating cytoskeletal rearrangement and intercellular communication in response to bioactive extract incorporation.

genAI

No artificial intelligence-based tools or generative AI technologies were used in this study. The entire content of the manuscript was originally prepared, reviewed, and approved by both authors.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

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References

1.Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol. 2008;17(12):1063-72. [Crossref]
2.Engebretsen KA, Johansen JD, Kezic S, Linneberg A, Thyssen JP. The effect of environmental humidity and temperature on skin barrier function and dermatitis. J Eur Acad Dermatol Venereol. 2016;30(2):223-49. [Crossref]
3.Fluhr JW, Feingold KR, Elias PM. Transepidermal water loss reflects permeability barrier status: validation in human and rodent in vivo and ex vivo models. Exp Dermatol. 2006;15(7):483-92. [Crossref]
4.Elias PM. Stratum corneum defensive functions: An integrated view. J Invest Dermatol. 2005;125(2):183-200. [Crossref]
5.Yao Z, Niu J, Cheng B. Prevalence of chronic skin wounds and their risk factors in an inpatient hospital setting in northern China. Adv Skin Wound Care. 2020;33(9):1-10. [Crossref]
6.Sawadkar P, Lali F, Garcia-Gareta E, Garrido BG, Chaudhry A, Matharu P, et al. Innovative hydrogels in cutaneous wound healing: current status and future perspectives. Front Bioeng Biotechnol. 2025;13:1454903. [Crossref]
7.Wang D, Cui F, Xi L, Tan X, Li J, Li T. Preparation of a multifunctional non-stick tamarind polysaccharide-polyvinyl alcohol hydrogel immobilized with a quorum quenching enzyme for maintaining fish freshness. Carbohydr Polym. 2023;302:120382. [Crossref]
8.Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Adv Mater. 2006;18(11):1345-1360. [Crossref]
9.Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. Polymer. 2008;49(8):1993-2007. [Crossref]
10.Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. J Adv Res. 2015;6(2):105-121. [Crossref]
11.Boateng J, Catanzano O. Advanced therapeutic dressings for effective wound healing—a review. J Pharm Sci. 2015;104(11):3653-3680. [Crossref]
12.Okay O. Macroporous copolymer networks. Prog Polym Sci. 2010;35(7):777-819.
13.Lozinsky VI. Cryogels: Applications in biotechnology and medicine. Gels. 2022;8(1):1-41.
14.Memic A, Abudula T, Mohammed HS, Joshi Navare K, Colombani T, Bencherif SA. Latest advances in cryogel technology for biomedical applications. Adv Ther. 2019;2(4):1800114. [Crossref]
15.Gun'ko VM, Savina IN, Mikhalovsky SV. Cryogels: Morphological, structural and adsorption characterization. Adv Colloid Interface Sci. 2017;246:1-39.
16.Verheij EWM, Coronel RE. Plant Resources of South-East Asia No. 2. Edible Fruits and Nut. Prosea, Bogor, Indonesia. 1992.
17.Perry LM. Medicinal plants of East and Southeast Asia: Attributed properties and uses. MIT Press. 1980.
18.Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2010;129(2):142-166. [Crossref]
19.Likhitwitayawuid K, Sritularak B, Benchanak K, Lipipun V, Mathew J, Schinazi RF. Phenolics with antiviral activity from Millettia erythrocalyx and Artocarpus lakoocha. Nat Prod Res. 2005;19(2):177-182. [Crossref]
20.Tsai MH, Liu JF, Chiang YC, Hu SC, Hsu LF, Lin YC, et al. Artocarpin, an isoprenyl flavonoid, induces p53-dependent or independent apoptosis via ROS-mediated MAPKs and Akt activation in non-small cell lung cancer cells. Oncotarget. 2017;8(17):28342-28358. [Crossref]
21.Palanuvej C, Ruangrungsi N, Vacharabhongsa P, Boonarkart C. Pharmacognostic study of Artocarpus lakoocha heartwood. Journal of Health Research. 2007;21(4):257-262.
22.Nazliniwaty N, Hanafiah OA, Pertiwi D, Muhammad M, Satria D. The activity of combination of ethanol extract of Artocarpus lacucha Buch.-Ham and Anredera cordifolia Steenis leaves to increase wound healing process on NIH-3T3 cell line. Open Access Maced J Med Sci. 2022;10:807-811. [Crossref]
23.Tanjung SA, Silalahi J, Reveny J. Wound Healing Activity of Nanoemulgel Containing Artocarpus lakoocha Roxb. Extract on Burns Model in Rat. Open Access Maced J Med Sci. 2022;10(A):725-733. [Crossref]
24.Hanafiah OA, Hanafiah DS, Dohude GA, Satria D, Putri MS, Harahap NIJ. Effects of 3% Mobe (Artocarpus lakoocha) leaf extract gel on the post-extraction socket: In-vivo study. Dental Journal (Majalah Kedokteran Gigi). 2024;57(2):102-109. [Crossref]
25.Senapong S, Puripattanavong J, Teanpaisan R. Anticandidal and antibiofilm activity of Artocarpus lakoocha extract. Songklanakarin Journal of Science & Technology. 2014;36(4).
26.Kaewkod T, Tragoolpua K, Tragoolpua Y. Encapsulation of Artocarpus lacucha Roxb. extract in alginate chitosan nanoparticles for inhibition of methicillin-resistant Staphylococcus aureus and bacteria causing skin diseases. Chiang Mai Journal of Science. 2016;43:946-958.
27.Teanpaisan R, Senapong S, Puripattanavong J. In vitro antimicrobial and antibiofilm activity of Artocarpus lakoocha (Moraceae) extract against some oral pathogens. Tropical Journal of Pharmaceutical Research. 2014;13(7):1149-1155. [Crossref]
28.Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965;16(3):144-158. [Crossref]
29.Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry. 1999;64(4):555-559. [Crossref]
30.Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200. [Crossref]
31.Razavi M, Hu S, Thakor AS. A collagen based cryogel bioscaffold coated with nanostructured polydopamine as a platform for mesenchymal stem cell therapy. J Biomed Mater Res A. 2018;106(8):2213-2228. [Crossref]
32.Canatar I, Özdaş S, Baydemir Peşint G. Phyllanthus emblica-Loaded Cryogels for Improved Wound Care: Characterization and In Vitro Studies. Macromolecular Materials and Engineering. 2024;309(4):2300404. [Crossref]
33.Van Tonder A, Joubert AM, Cromarty AD. Limitations of the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes. 2015;8(1):47. [Crossref]
34.Foo JB, Ng LS, Lim JH, Tan PX, Lor YZ, Loo JSE, et al. Induction of cell cycle arrest and apoptosis by copper complex Cu (SBCM) 2 towards oestrogen-receptor positive MCF-7 breast cancer cells. RSC Advances. 2019;9(32):18359-18370. [Crossref]
35.Yılmaz Ü, Seyhan MF. Effect of Ellagic Acid and Cryptotanshinone on Cell Viability/Cytotoxicity, Metastasis, and Oxidative Stress in Triple-Negative Breast Cancer Cells. Experimed. 2024;14(1):46-53. [Crossref]
36.Panichakul T, et al. Phytochemical constituents and biological activities of Artocarpus lakoocha Roxb. Natural Product Research. 2020;34(7):961-964.
37.Tarbiat B. Antioxidant activity of phenolic compounds from Artocarpus lakoocha and their potential health benefits. Journal of Food Science and Technology. 2018;55(7):2625-2633.
38.Ahamad J, Amin S, Mir SR, Ahmad MA, Ahmad W, Anwar F. Antioxidant and antimicrobial activity of heartwood extracts of Artocarpus lakoocha Roxb. Journal of Herbal Medicine. 2020;21:100345.
39.Nazliniwaty N, Dalimunthe A, Sitorus P, Ahamad J. Phytochemical screening and antioxidant activity of Artocarpus lakoocha Roxb. extract. Pharmacognosy Journal. 2021;13(3):664-670.
40.Sitorus P, Dalimunthe A, Nazliniwaty N. Evaluation of antioxidant properties of Artocarpus lakoocha extracts using DPPH and ABTS assays. Journal of Applied Pharmaceutical Science. 2022;12(6):35-41.
41.Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of Etlingera elatior. Journal of Food Composition and Analysis. 2009;22(5):388-393.
42.Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 2011;8(1). [Crossref]
43.Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-7352. [Crossref]
44.Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;2(4):152-159. [Crossref]

45.Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects. Journal of Functional Foods. 2015;18:820-897. [Crossref]
46.Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry. 2015;97:55-74. [Crossref]
47.Baur JA, Sinclair DA. Therapeutic potential of resveratrol: The in vivo evidence. Nature Reviews Drug Discovery. 2006;5(6):493-506. [Crossref]
48.Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance & Infection Control. 2019;8(1):1-28. [Crossref]
49.Hankittichai P, Buacheen P, Pitchakarn P, Na Takuathung M, Wikan N, Smith DR, et al. Artocarpus lakoocha extract inhibits LPS-induced inflammatory response in RAW 264.7 macrophage cells. International Journal of Molecular Sciences. 2020;21(4):1355. [Crossref]
50.Choi EJ, et al. Oxyresveratrol promotes cutaneous wound healing in rats. Phytotherapy Research. 2014;28(3):441-447.
51.Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences. 2008;97(8):2892-2923. [Crossref]
52.Kamoun EA, Kenawy E-RS, Chen X. A review on polymeric hydrogel membranes for wound dressing applications. Materials Science and Engineering: C. 2017;77:1301-1312.
53.Gopi S, George R, Thomas S. Polymeric biomaterials in wound healing: An overview. Polymers for Advanced Technologies. 2016;27(6):844-854.
54.Canatar İ, Zenger O, Özdaş S, Baydemir Peşint G. Pterostilbene loaded poly (vinyl alcohol)-gelatin cryogels as potential bioactive wound dressing material. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2023;111(6):1259-1270. [Crossref]
55.Górska A, Krupa A, Majda D, et al. Poly(vinyl alcohol) cryogel membranes loaded with resveratrol as potential active wound dressings. Materials & Applications in Medicine and Engineering. 2021. [Crossref]
56.Kolósova OY, Vasil'ev VG, Novikov IA, Sorokina EV, Lozinsky VI. Cryostructuring of polymeric systems: Properties and microstructure of PVA cryogels formed in the presence of phenolic compounds. Polymers. 2024;16(5):675. [Crossref]
57.He Q, Feng T, Xie Y, Swamiappan S, Zhou Y, Zhou Y, et al. Recent Advances in the Development and Application of Cell-Loaded Collagen Scaffolds. International Journal of Molecular Sciences. 2025;26(9):4009. [Crossref]
58.Zheng Y, Liu H. Influence of phenolic compounds on cryogel pore morphology. Cryobiology Journal. 2022.
59.Sezen S, et al. Macroporous cryogels in biomaterials: Structure-function relationships. Journal of Biomaterials Science. 2021.
60.Medeiros SF, Silva FE. Hydrogel dressings: A review. Journal of Advanced Research. 2021;6(2):105-121.
61.Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. International Journal of Inflammation. 2019;2019:3706315. [Crossref]